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(54) Title: TREATMENT FOR ASTHMA (57) Abstract A method for the treatment of asthma is disclosed. The method comprises administration of an antibody, polypeptide or other molecule recognizing VLA-4, a protein expressed on the surface of certain leukocytes such as eosinophils.		

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TREATMENT FOR ASTHMA

FIELD OF THE INVENTION

The present invention relates to a treatment for
5 asthma. More particularly, this invention relates to the
use of antibodies recognizing Very Late Antigen-4 (VLA-4),
a ligand on certain leukocytes for the endothelial cell
receptor Vascular Cell Adhesion Molecule-1 (VCAM-1), in
the treatment of asthma.

10 BACKGROUND OF THE INVENTION

Asthma is a condition of the respiratory tract
characterized by widespread, reversible narrowing of the
airways (bronchoconstriction) and increased sensitivity
(hyperresponsiveness) of the airways to a variety of
15 stimuli. The familiar symptomology of asthma, i.e.,
coughing, wheezing, chest tightness, dyspnea, is caused by
airway smooth muscle contraction, increased bronchial
mucus secretion, and inflammation. Though seldom fatal,
asthma has been estimated to affect 10-20% of school-aged
20 children around the world, and hospital admissions for
asthma in children have increased dramatically in recent
years, one survey for the United States indicating that
hospital admissions for children under 15 with asthma
increased by at least 145% between 1970 and 1984. (See,
25 M.R. Sears, 1990 [1].) Overall, it is estimated that 10
million Americans (4% of the population) have asthma, and
some \$4 billion is spent in treatment per year. (L.K.
Altman, 1991 [2]; C. Starr, 1991 [3].)

The causes of asthma are not completely
30 understood, however the study of agents that trigger acute
asthmatic episodes supports the theory that asthma is an
immunological reaction by a subject in response to
specific allergens of the subject's environment. These
"triggers" exacerbate asthma by causing transient

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enhancement of airway hyperresponsiveness. Triggers that have been found to induce airway hyperresponsiveness include inhaled allergens, inhaled low molecular weight agents to which the subject has become sensitized (e.g., by occupational exposure), viral or mycoplasma respiratory infections, and oxidizing gases such as ozone and nitrogen dioxide. These "inducing" triggers can be distinguished from "inciting" triggers of bronchospastic episodes which include exercise, cold air, emotional stress, pharmacological triggers, inhaled irritants. The common feature of inducing triggers is that they are associated with airways inflammation; inciting triggers produce smooth muscle contractions (bronchospasms) which depend on the underlying degree of hyperresponsiveness, rather than increasing airways responsiveness themselves. (See, D.W. Cockcroft, 1990 [4].)

The recognition that airways inflammation is a cause of transient (acute) and also persistent airway hyperresponsiveness has had an impact on the treatment of asthma sufferers. Early treatments for asthma focused on bronchoconstriction and led to the development of many effective bronchodilator drugs. The most commonly prescribed were beta₂-adrenoceptor agonists (epinephrine, isoproterenol, albuterol, salmeterol, etc.), xanthines (caffeine, theophylline, etc.) and cholinceptor antagonists (atropine, acetylcholine, etc.). More recently, however, anti-inflammatory drugs have begun to replace bronchodilators as first-line treatments for asthma. Commonly prescribed anti-inflammatory agents for asthma include disodium cromoglycate (DSCG), nedocromil sodium, antihistamines such as ketotifen, and

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corticosteroids such as prednisolone. (See, F.M.C. Cuss, 1990 [5] and P.M. O'Byrne, 1990 [6].)

The inflammatory response in asthma is typical for tissues covered by a mucosa and is characterized by
5 vasodilation, plasma exudation, recruitment of
inflammatory cells such as neutrophils, monocytes, macrophages, lymphocytes and eosinophils to the sites of inflammation, and release of inflammatory mediators by
resident tissue cells (e.g., mast cells) or by migrating
10 inflammatory cells. (J.C. Hogg, 1990 [7].) In allergen-induced asthma, sufferers often exhibit a dual response to exposure to an allergen --an "early phase" response beginning immediately after exposure and lasting until 1-2 hours after exposure, followed by a "late phase" response
15 beginning about 3 hours after exposure and lasting sometimes until 8-10 hours or longer after exposure. (D.W. Cockcroft, 1990 [4].) Late phase response in allergen-induced asthma and persistent hyperresponsiveness have been associated with the recruitment of leukocytes,
20 and particularly eosinophils, to inflamed lung tissue. (W.M. Abraham et al., 1988 [8].) Eosinophils are known to release several inflammatory mediators, e.g., 15-HETE, leukotriene C₄, PAF, cationic proteins, eosinophil peroxidase. (K.F. Chung, 1990 [9].)

25 Many of the drugs used to treat asthma have been found to block or neutralize the effects of the release of inflammatory mediators which regulate the inflammatory response. For example, beta₂-adrenoceptor agonists and DSCG are potent stabilizers of mast cells, which are
30 capable of releasing many mediators, including histamine, prostaglandins, leukotrienes, platelet activating factor (PAF), and chemotactic factors for neutrophils and

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eosinophils; corticosteroids, as another example, complex with steroid hormone receptors, which leads to the synthesis of proteins, such as lipocortins, that produce anti-inflammatory effects. (F.M.C. Cuss, 1990 [5].)

5 Although known asthma medications have some effect on leukocyte recruitment into the lung (W.M. Abraham et al., 1990 [8]), none of these drugs is effective to directly block migration of leukocytes into inflamed tissues.

10 Inflammatory leukocytes are recruited to sites of inflammation by cell adhesion molecules that are expressed on the surface of endothelial cells and which act as receptors for leukocyte surface proteins or protein complexes. Eosinophils have recently been found to
15 participate in three distinct cell adhesion pathways to vascular endothelium, binding to cells expressing intercellular adhesion molecule-1 (ICAM-1), endothelial cell adhesion molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1). (P.F. Weller et al., 1991
20 [10]; G.M. Walsh et al., 1991 [11]; B.S. Bochner et al., 1991 [12]; and A. Dobrina et al., 1991 [13].) VCAM1 binds to the $\alpha_4\beta_1$ integrin, VLA-4, which is expressed on various lymphoid cells, including eosinophils (Weller et al., 1991 [10]; Elices et al. 1990 [14]). That eosinophils express
25 VLA-4 differentiates them from other inflammatory cells such as neutrophils, which bind to ELAM-1 and ICAM-1 but not VCAM-1.

 The VLA-4-mediated adhesion pathway was investigated in an asthma model to examine the possible
30 role of VLA-4 in leukocyte recruitment to inflamed lung tissue. It has now been discovered that administering anti-VLA-4 antibody inhibits both the late phase response

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and airway hyperresponsiveness in allergic sheep. Surprisingly, administration of anti-VLA-4 led to a reduction in the number of both neutrophils and eosinophils in the lung at 4 hours after allergen challenge, even though both cells have alternate adhesion pathways by which they can be recruited to lung tissues. Also surprisingly, inhibition of hyperresponsiveness in the treated sheep was observed which continued to 1 week, even though infiltration of leukocytes, including neutrophils and eosinophils, was not significantly reduced over time.

SUMMARY OF THE INVENTION

The present invention provides novel methods for the treatment of asthma and further provides new pharmaceutical compositions useful in the treatment of asthma. In particular, the present invention provides a method comprising the step of administering to an asthma sufferer an effective amount of an anti-VLA-4 antibody, such as monoclonal antibody HP1/2. The anti-VLA-4 antibody is advantageously administered in vivo to a patient with chronic allergen-induced asthma, and serves to inhibit late phase response to allergens and to attenuate airway hyperresponsiveness.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph depicting the effect of monoclonal antibody HP1/2 (intravenous) on the response to allergen (Ascaris suum antigen) in dual responder allergic sheep. Percentage change in specific lung resistance (SR_L) is measured over time post allergen challenge. Asterisks indicate statistically significant results.

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Figure 2 is a graph depicting plasma concentration of monoclonal antibody HP1/2 (intravenous) in sheep, measured over time after initial administration.

Figure 3 is a graph depicting the effect of monoclonal antibody HP1/2 (intravenous) on airway hyperresponsiveness in dual responder sheep. Airway responsiveness, measured in breath units (BU) of cumulative breaths of a 1% weight/volume carbachol solution (a known bronchoconstrictor) that increases specific lung resistance 400% over the value obtained using diluent alone. Asterisks indicate statistically significant results.

Figure 4 is a series of four graphs showing the total cells and the levels of different leukocytes (lymphocytes, neutrophils, and eosinophils) detected by bronchoalveolar lavage in allergic sheep challenged with Ascaris suum antigen alone and after pretreatment with monoclonal antibody HP1/2 (intravenous). Total cells, and the percentage of total cells that were lymphocytes or neutrophils or eosinophils, were measured at 4-hour, 8-hour, 24-hour, 48-hour and 1-week time points post allergen challenge.

Figure 5 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg, aerosol) on the response to allergen (Ascaris suum antigen) in dual responder allergic sheep. Percentage change in specific lung resistance (SR_L) is measured over time post allergen challenge. Asterisks indicate statistically significant results.

Figure 6 is a graph depicting the effect of monoclonal antibody HP1/2 (16 mg, aerosol) and 1E6 (16 mg, aerosol) on airway hyperresponsiveness in dual responder

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sheep. Airway responsiveness, measured in breath units (BU) of cumulative breaths of a 1% weight/volume carbachol solution (a known bronchoconstrictor) that increases specific lung resistance 400% over the value obtained using diluent alone. Asterisks indicate statistically significant results.

DETAILED DESCRIPTION OF THE INVENTION

The technology for producing monoclonal antibodies is well known. Briefly, an immortal cell line (typically myeloma cells) is fused to lymphocytes (typically splenocytes) from a mammal immunized with whole cells expressing a given antigen, e.g., VLA-4, and the culture supernatants of the resulting hybridoma cells are screened for antibodies against the antigen. (See, generally, Kohler et al., 1975 [15].)

Immunization may be accomplished using standard procedures. The unit dose and immunization regimen depend on the species of mammal immunized, its immune status, the body weight of the mammal, etc. Typically, the immunized mammals are bled and the serum from each blood sample is assayed for particular antibodies using appropriate screening assays. For example, anti-VLA-4 antibodies may be identified by immunoprecipitation of ¹²⁵I-labeled cell lysates from VLA-4-expressing cells. (See, Sanchez-Madrid et al. 1986 [16] and Hemler et al. 1987 [17].) Anti-VLA-4 antibodies may also be identified by flow cytometry, e.g., by measuring fluorescent staining of Ramos cells incubated with an antibody believed to recognize VLA-4 (see, Elices et al., (1990) [14]). The lymphocytes used in the production of hybridoma cells typically are isolated from immunized mammals whose sera have already tested positive

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for the presence of anti-VLA-4 antibodies using such screening assays.

Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium").

Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using 1500 molecular weight polyethylene glycol ("PEG 1500"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridomas producing a desired antibody are detected by screening the hybridoma culture supernatants. For example, hybridomas prepared to produce anti-VLA-4 antibodies may be screened by testing the hybridoma culture supernatant for secreted antibodies having the ability to bind to a recombinant α_4 -subunit-expressing cell line, such as transfected K-562 cells (see, Elices et al. [14]).

To produce anti VLA-4-antibodies, hybridoma cells that tested positive in such screening assays were cultured in a nutrient medium under conditions and for a time sufficient to allow the hybridoma cells to secrete the monoclonal antibodies into the culture medium. Tissue culture techniques and culture media suitable for hybridoma cells are well known. The conditioned hybridoma culture supernatant may be collected and the anti-VLA-4 antibodies optionally further purified by well-known methods.

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Alternatively, the desired antibody may be produced by injecting the hybridoma cells into the peritoneal cavity of an unimmunized mouse. The hybridoma cells proliferate in the peritoneal cavity, secreting the antibody which accumulates as ascites fluid. The antibody may be harvested by withdrawing the ascites fluid from the peritoneal cavity with a syringe.

Several anti-VLA-4 monoclonal antibodies have been previously described (see, e.g., Sanchez-Madrid et al., 1986 [16]; Hemler et al. (1987) [17]; Pulido et al. (1991) [19]). For the experiments herein, an anti-VLA-4 monoclonal antibody designated HP1/2 (obtained from Biogen, Inc., Cambridge, MA) was used. The variable regions of the heavy and light chains of the anti-VLA-4 antibody HP1/2 have been cloned, sequenced and expressed in combination with constant regions of human immunoglobulin heavy and light chains. Such a chimeric HP1/2 antibody is similar in specificity and potency to the murine HP1/2 antibody, and may be useful in methods of treatment according to the present invention. Similarly, humanized recombinant anti-VLA-4 antibodies may be useful in these methods. The HP1/2 V_H DNA sequence and its translated amino acid sequences are set forth in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The HP1/2 V_K DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

Monoclonal antibodies such as HP1/2 and other anti-VLA-4 antibodies (e.g., Mab HP2/1, HP2/4, L25, P4C2) capable of recognizing the α chain of VLA-4 will be useful in the present invention. It is most preferred that the antibodies will recognize the B1 or B2 epitopes of the VLA- α_4 chain (see, Pulido et al. (1991) [19]). While not

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wishing to be bound by one scientific theory, anti-VLA-4 antibodies used according to the method of the present invention may specifically inhibit, at least for an initial period following allergen challenge, the migration of VLA-4-expressing leukocytes to inflamed sections of the lung. This inhibition of VLA-4 leukocyte migration could, in turn, prevent secondary pathological effects of leukocyte infiltration, e.g., release of toxic substances, inducement of soluble inflammatory cell mediators, release or inducement of leukocyte chemotactic agents (such as neutrophil chemotactic factors), etc. As a result, late phase response to the allergen and continuing hypersensitivity of the airways may be attenuated. Alternatively, the anti-VLA-4 antibodies may attenuate signal transduction necessary for the release of inflammatory mediators and/or cell chemotactic agents.

The method of the present invention comprises administering to a mammal suffering from allergic asthma a composition comprising an anti-VLA-4 antibody. The examples below set forth the results observed in asthmatic sheep. However, the similarity between physiological responses and pharmacological effects in sheep and in humans has been documented (see, e.g., W.M. Abraham, 1989 [20]); and similarities between sheep and other animal asthma models (rabbits, squirrel monkeys, guinea pigs, and sensitized dogs) have been noted (see, e.g., W.M. Abraham et al., 1988 [8]). Accordingly, the results reported herein will be relevant and applicable to, and the method claimed will be useful in, any mammal, including humans, suffering from allergic asthma.

The anti-VLA-4 antibody administered in accordance with the present invention may be administered prophylactically, before exposure to an asthma-inducing

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allergen. Beneficial effects will also be obtained if the antibody is administered at the time of or immediately after allergen exposure, between early phase and late phase response to attenuate the severity of late phase response, or at any time following allergen exposure to reduce or eliminate airway hyperresponsiveness.

The anti-VLA-4 antibody can be administered in the form of a composition comprising an anti-VLA-4 antibody and a pharmaceutically acceptable carrier.

10 Preferably, the composition will be in a form suitable for intravenous injection. Also contemplated are antibody compositions in the form of a sterile aqueous or phosphate-buffered saline solution which can be nebulized (atomized) and breathed directly into the lungs by the

15 asthma sufferer, e.g., using an inhaler. Dosages will vary depending on the sensitivity of the asthma sufferer to particular allergens, the concentration of allergen on exposure and frequency/duration of exposure(s), the proposed mode of administration (e.g., injection or

20 inhalation), the desired plasma level of antibody, the effectiveness of a particular antibody or combination of antibodies in suppressing airway responsiveness, the clearance rate or half-life of the antibody composition, and other such factors familiar to physicians experienced

25 in the treatment of allergic asthma. In general, dosages will be calculated and adjusted to maintain a plasma level of antibody in the range of from 1-1000 $\mu\text{g/ml}$, although higher or lower dosages may be indicated with consideration to the age, sensitivity, tolerance, and

30 other characteristics of the patient, the acuteness of the flareup, the history and course of the disease, and other similar factors routinely considered by an attending physician. Depending on the potency and half-life of the

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antibody employed, it is preferred to use from about 0.05 mg/kg to 5.0 mg/kg of antibody, most preferably from 0.5 to 2.0 mg/kg of antibody, based on the weight of the patient receiving treatment.

5 Suitable pharmaceutical carriers include, e.g., sterile saline and physiological buffer solutions. Phosphate buffered saline (PBS) is preferred for inhalant administration. The pharmaceutical compositions may additionally be formulated to control the release of the
10 active ingredients or to prolong their presence in the patient's system. Numerous suitable drug delivery systems are known for this purpose and include, e.g., hydrogels, hydroxymethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like.

15 It will also be recognized that for the purposes of the present invention, antibodies capable of binding to the α_4 subunit of VLA-4 must be employed. It is preferred that monoclonal antibodies be used.

 In addition to naturally produced antibodies,
20 suitable recombinant antibodies capable of binding to VLA-4 may alternatively be used. Such recombinant antibodies include antibodies produced via recombinant DNA techniques, e.g., by transforming a host cell with a suitable expression vector containing DNA encoding the
25 light and heavy immunoglobulin chains of the desired antibody, and recombinant chimeric antibodies, wherein some or all of the hinge and constant regions of the heavy and/or the light chain of the anti-VLA-4 antibody have been substituted with corresponding regions of an
30 immunoglobulin light or heavy chain of a different species (i.e., preferably the same species as the asthma sufferer being treated, to minimize immune response to the

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administered antibody). (See, e.g., P.T. Jones et al., 1986 [21], E.S. Ward et al., 1989 [22], and U.S. Patent 4,816,397 (Boss et al.) [23], all incorporated herein by reference.)

5 Furthermore, VLA-4-binding fragments of anti-VLA-4 antibodies, such as Fab, Fab', F(ab')₂, and F(v) fragments; heavy chain monomers or dimers; light chain monomers or dimers; and dimers consisting of one heavy chain and one light chain are also contemplated herein.

10 Such antibody fragments may be produced by chemical methods, e.g., by cleaving an intact antibody with a protease, such as pepsin or papain, or via recombinant DNA techniques, e.g., by using host cells transformed with truncated heavy and/or light chain genes. Heavy and light

15 chain monomers may similarly be produced by treating an intact antibody with a reducing agent such as dithiothreitol or β -mercaptoethanol or by using host cells transformed with DNA encoding either the desired heavy chain or light chain or both.

20 Also, from the foregoing discussion it will be apparent that other polypeptides and molecules which inhibit or block VLA-4-mediated binding will be effective in the treatment of asthma in the same manner as anti-VLA-4 antibodies. For example, a soluble form of VCAM-1 (an

25 endothelial cell receptor for VLA-4) or a fragment thereof may be administered to compete for the VLA-4 binding site, thereby leading to effects similar to the administration of anti-VLA-4 antibodies. Small molecules such as oligosaccharides that mimic the binding domain of an VLA-4

30 ligand and fit the receptor domain of VLA-4 may also be employed. (See, J.J. Devlin et al., 1990 [24], J.K. Scott and G.P. Smith, 1990 [25], and U.S. Patent 4,833,092

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(Geysen) [26], all incorporated herein by reference.) The use of such VLA-4-binding polypeptides or molecules that effectively inhibit late phase response or airway hyperresponsiveness in allergic subjects is contemplated herein as an alternative method for treatment of asthma.

It is also contemplated that anti-VLA-4 antibodies may be used in combination with other antibodies having a therapeutic effect on airway responsiveness. For instance, to the extent that the beneficial effects reported herein are due to the inhibition of leukocyte recruitment to VCAM-1-expressing endothelium, combinations of anti-VLA-4 antibodies with other antibodies that interfere with the adhesion between leukocyte antigens and endothelial cell receptor molecules may be advantageous. For example, in addition to the use of anti-VLA-4 antibodies in accordance with this invention, the use of anti-ELAM-1 and/or anti-ICAM-1 antibodies may be advantageous. [See, Gundel et al. (1991) [27]; Wegner et al. (1990) [28].]

When formulated in the appropriate vehicle, the pharmaceutical compositions contemplated herein may be administered by any suitable means such as orally, intraesophageally or intranasally, intrabronchially (local treatment, e.g., via bronchoscope), as well as subcutaneously, intramuscularly, intravenously, intra-arterially, or parenterally. Ordinarily administration via inhalation is preferred.

EXAMPLES

Experiments were performed essentially as described by Abraham et al. [8]. Briefly, allergic sheep having natural allergic cutaneous reaction to 1:1000 or 1:10,000 dilutions of Ascaris suum extract (Greer Diagnostics, Lenoir NC) were tested, and sheep

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demonstrating both early and late phase airway response ("dual responders") to inhalation challenge with Ascaris suum antigen were selected. To measure breathing mechanics and physical changes in the airways, the sheep

5 were restrained in a prone position with heads immobilized. A balloon catheter was advanced through one nostril under topical anesthesia with 2% lidocaine solution to the lower esophagus, and a cuffed endotracheal tube was advanced through the other nostril (using a

10 flexible fiberoptic bronchoscope as a guide) for the measurement of airway mechanics and during aerosol challenges. Pleural pressure was estimated with the esophageal balloon catheter (filled with 1 ml of air) positioned 5-10 cm from the gastroesophageal junction. In

15 this position, end expiratory pleural pressure ranged between -2 and -5 cm H₂O. Once the balloon was placed, it was secured so that it remained in position for the duration of the experiment. Lateral pressure in the trachea was measured with a sidehole catheter, (inner

20 diam. 2.5 mm) advanced through and positioned distal to the tip of the endotracheal tube. Transpulmonary pressure (the difference between tracheal and pleural pressure) was measured with a differential pressure transducer catheter system (MP45, Validyne, Northridge,

25 CA). The pressure transducer catheter system showed no phase shift between pressure and flow to a frequency of 9 Hz. Pulmonary resistance (R_L) was measured by connecting the proximal end of the endotracheal tube to a Fleisch pneumotachograph (Dyna Sciences, Blue Bell PA).

30 Signals indicating flow and transpulmonary pressure were recorded on an oscilloscope recorder (Model DR-12; Electronics for Medicine, White Plains, NY) linked to a

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computer for automatic calculation of pulmonary resistance (R_L) from transpulmonary pressure, respiratory volume (obtained by digital integration) and flow by the mid-volume technique, analyzed from 5-10 breaths. Thoracic gas volume (V_g) was measured immediately after determination of R_L in a constant volume body plethysmograph. Specific lung resistance (SR_L) was calculated from these values ($SR_L = V_g \times R_L$).

Airway responsiveness was determined by performing dose response curves to inhaled carbachol. The dose response curves were plotted using measurements of SR_L taken immediately after inhalation of buffer (PBS) alone and after each consecutive administration of 10 breaths of increasing concentrations of carbachol in PBS. The concentrations of carbachol were 0.25%, 0.5%, 1.0%, 2.0% and 4.0% wt/vol in PBS. The provocation test was discontinued when SR_L increased over 400% from the post-PBS value or after the highest carbachol concentration had been administered. Airway responsiveness was determined by calculating from the dose response curves the cumulative carbachol dose in breath units (BU) that increased specific lung resistance 400% over the post buffer value ($PD_{400\%}$). One breath unit was defined as one breath of a 1% wt/vol carbachol solution. Thus, the greater the suppression of airway hyper-responsiveness, the greater the number of breath units would be required before observing the same bronchoconstriction as seen in the controls.

Each sheep was subjected to a trial as a control in which a placebo (PBS without additive) was used as a pretreatment 30 minutes before allergen challenge with Ascaris suum antigen (Greer Diagnostics, Lenoir, NC).

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Subsequently, the sheep were subjected to an identical trial, except that 1 mg/kg of monoclonal antibody HP1/2 was administered to each sheep 30 minutes prior to antigen challenge. The placebo (buffer control or isotope-matched antibody (1E6, anti-LFA3) control) and HP1/2 compositions were administered by intravenous injection. The HP1/2 composition (and the 1E6 control) was prepared by diluting pure antibody obtained from a hybridoma (Biogen, Inc., Cambridge MA) in sterile, endotoxin-free PBS and adjusting to deliver 1 mg/kg antibody based on the weight of each sheep. The antigen solution was delivered as an aerosol using a disposable medical nebulizer (RAINDROP®, Puritan Bennett, Lenexa, KS) that provided an aerosol with a mass median aerodynamic diameter of 3.2 μ M (geometric SD 1.9) as determined by an Andersen cascade impactor. The Ascaris suum extract was diluted in PBS to a concentration of 82,000 Protein Nitrogen Units(PNU)/ml. The output of the nebulizer was directed into a plastic T-tube, one end of which was connected to the inspiratory port of a Harvard respirator. A dosimeter connected to the nebulizer consisting of a solenoid valve and a 20 psi compressed air source and the solenoid valve was activated at the beginning of the inspiratory cycle of the Harvard respirator for one second. The aerosol delivered at a tidal volume of 500 ml and a rate of 20 breaths per min. for 20 min. Each sheep was challenged with an equivalent dose of antigen (400 breaths) in the control and HP1/2 trials. Carbachol aerosols for the dose response curves were also generated by nebulizer as described above.

For cellular analysis, bronchoalveolar lavage (BAL) was performed on each sheep. The distal tip of the specially designed 80 cm fiberoptic bronchoscope was gently wedged into a randomly selected subsegmental

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bronchus. Lung lavage was performed by slow infusion and gentle aspiration of 3 x 30 ml of PBS (pH 7.4) at 39° C, using 30 ml syringes attached to the working channel of the bronchoscope. The lavage return was collected, strained through gauze to remove mucus and then centrifuged at 420 g for 15 min. Supernatant was decanted, and the cells were resuspended in PBS. An aliquot of the suspension was transferred to a hemocytometer chamber to estimate total cells. Total viable cells were estimated by trypan blue exclusion. A second aliquot of the cell suspension was spun in a cytospin (600 rpm for 10 minutes) and stained by Wright-Giemsa and observed at 100X to identify cell populations. 500 cells per slide were characterized to establish the differential cell counts. Cells characterized included epithelial cells, macrophages, basophils, monocytes and unidentifiable cells (grouped into a category termed "others"), in addition to lymphocytes, neutrophils and eosinophils.

Plasma level of antibody and white blood cell counts were determined from venous blood samples (approx. 5 ml) from peripheral leg vein or jugular vein.

Example 1

An airway challenge trial using eight dual responder allergic sheep was conducted according to the foregoing protocols. Baseline (BSL) airway responsiveness (PD_{400%}) was established 2-3 days prior to antigen challenge and a baseline bronchoalveolar lavage (BAL) was performed one day prior to challenge. On challenge day, baseline values for specific lung resistance (SR_L) was measured, then the sheep were administered buffer (control) or HP1/2 by injection. After this first

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administration ("treatment"), SR_L was measured, and 30 min. after treatment, the sheep were challenged with Ascaris suum antigen. SR_L was measured immediately after challenge, hourly from 1-6 hours following challenge, every half-hour from 6.5 hours to 8 hours, and also at 24 hours, 48 hours and 1 week (i.e., 168 hours) after antigen challenge. BALs were performed following SR_L measurements at 4, 8, 24 and 48 hours and at 1 week. For these studies, peripheral blood was drawn and total white blood cell counts and assessment of cell populations were taken before treatment (baseline), immediately after challenge, and at 1, 2, 3, 4, 6, 8, 24 and 48 hours, and 1 week after challenge. The results of this trial are shown in the figures:

Figure 1 shows the effect of HP1/2 treatment on antigen-induced airway responses in the subject sheep. HP1/2 treatment resulted in significant (indeed, virtually complete) inhibition of the late phase response experienced by the controls.

Figure 2 is a graph of plasma concentration of HP1/2 in $\mu\text{g/ml}$ in the treated subjects, measured immediately following antigen challenge and then at 1, 2, 3, 4, 6, 8, 24 and 48 hours after challenge. After equilibration, the antibody concentration settled to a concentration of approximately 20 $\mu\text{g/ml}$, which concentration was maintained out to the 48-hour point.

Figure 3 is a graph showing the effect of HP1/2 treatment on airway responsiveness. At 24, 48, and 1 week after antigen challenge, treated subjects showed significant decrease in airway responsiveness. Even at 2 weeks after antigen challenge, treated subjects continued to show decreases in airway responsiveness. The fact that

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the virtually complete inhibitory effect of the antibody lasted out to 1 week is especially surprising and encouraging in terms of the therapeutic value of the treatment.

5 Figure 4 is a series of graphs illustrating the results of BALs performed at 4, 8, 24 and 48 hours after antigen challenge, and at 1 week after antigen challenge. The results show no significant changes over controls in total cells recovered from treated subjects. However,
10 treated subjects showed reduced levels of both neutrophils and eosinophils at the 4-hour time point after challenge. This is somewhat surprising, given that the administration of anti-VLA-4 would not be expected to influence neutrophil recruitment, since neutrophils do not express
15 VLA-4. Also, both neutrophils and eosinophils express alternative ligands involved in adhesion to endothelium; both types of cells have been shown to bind to endothelial cells via the LFA-1/ICAM-1 pathway and the CDX/ELAM-1 pathway.

20 Similar therapeutic effects with the anti-VLA-4 antibody HP1/2 were observed when the subjects were treated with HP1/2 antibody 2 hours after antigen challenge as opposed to 30 minutes prior to challenge as described above. The effect of HP1/2 was dose-dependent.
25 For example, reducing the dose to 0.2 mg/kg was not sufficient to protect against the late response. For the antigen challenge studies in which 1E6 (anti-LFA3) was used as an isotope-matched control antibody for the HP1/2 treatment, no effect on the early or late response was
30 observed using 1E6 in a control trial. The 1E6-2C12 hybridoma cell line producing the 1E6 antibody has been deposited as ATCC HB 10693.

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Example 2

A subsequent experiment was performed to investigate the efficacy of aerosol delivery of the antibody. The trials were performed essentially as described above, except that two sheep were used, and the HP1/2 was delivered via nebulizer in the form of an aerosol (dose = 8 mg HP1/2 per animal, administered 1/2 hour prior to antigen challenge).

In control sheep (receiving placebo), the late phase response was characterized by an average increase in SR_L of 126% of the baseline value, whereas when the sheep were treated with the anti-VLA-4 antibody, average rise in SR_L was 26% of baseline. These results amount to approximately 80% inhibition of late phase response. The results also indicated about 70% inhibition of airway responsiveness at 24 hours. From this trial, it is apparent that inhalant delivery of the antibody may be used to obtain the benefits of this invention.

These data were confirmed and extended to 5 sheep with controls (isotype-matched 1E6 (anti-LFA3) antibody control) using a 16 mg/kg aerosol dose of HP1/2 (n=5) or 1E6 (n=4). Figures 5 and 6 show that treatment with HP1/2 aerosol at this dose 30 minutes before antigen challenge is also effective in blocking the late response and airway hyperresponsiveness. HP1/2 aerosol treatment resulted in significant (indeed, virtually complete) inhibition of the late phase response experienced by the 1E6 controls. 1E6 aerosol treatment was without effect. Although comparable protection was achieved in both the intravenous and aerosol trials, the protection afforded by HP1/2 in the aerosol trials was achieved without detectable blood levels of the drug. This effect of HP1/2

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is specific because the same dose of 1E6 had no protective effect (e.g., 1E6 treated animals showed a significant fall in PC₄₀₀, whereas HP1/2 blocked the effect). The differences in the physiological responses between HP1/2 and 1E6 are not the result of deficiencies in total WBC or differential counts between the groups. Total WBC and differential in both the HP1/2 and 1E6 groups showed a pattern of responses similar to those seen in the intravenous trials.

10 The foregoing examples are intended as an illustration of the method of the present invention and are not presented as a limitation of the invention as claimed hereinafter. From the foregoing disclosure, numerous modifications and additional embodiments of the invention will be apparent to those experienced in this art. For example, actual dosage used, the type of antibody or antibody fragment used, mode of administration, exact composition, time and manner of administration of the treatment, and many other features
15 all may be varied without departing from the description above. All such modifications and additional embodiments are within the contemplation of this application and
20 within the scope of the appended claims.

- 23 -

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The foregoing documents are incorporated herein by reference.

-26-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Lobb, Roy R.
- (ii) TITLE OF INVENTION: Treatment for Asthma
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Allegretti & Witcoff, Ltd.
 - (B) STREET: 10 South Wacker Drive, Suite 3000
 - (C) CITY: Chicago
 - (D) STATE: IL
 - (E) COUNTRY: US
 - (F) ZIP: 60606
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT
 - (B) FILING DATE: 12 January 1993
 - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: McNicholas, Janet M.
 - (B) REGISTRATION NUMBER: 32,918
 - (C) REFERENCE/DOCKET NUMBER: 92,307-A; D002 CIP PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 312-715-1000
 - (B) TELEFAX: 312-715-1234

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 360 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

-27-

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "pBAG159 insert: HP1/2 heavy chain variable region; amino acid 1 is Glu (E) but Gln (Q) may be substituted"

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(A) NAME/KEY: CDS

(B) LOCATION: 1..360

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Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr	
21 26 31	
ATG CAC TGG GTG AAG CAG AGG CCT GAA CAG GGC CTG GAG TGG ATT GGA	144
Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly	
36 41 46	
AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC CCG AAG TTC CAG	192
Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln	
51 56 61	
GTC AAG GCC ACT ATT ACA GCG GAC ACG TCC TCC AAC ACA GCC TGG CTG	240
Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu	
66 71 76 81	
CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCC GTC TAC TAC TGT GCA	288
Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala	
86 91 96	
GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC TTC TGG GGC CAA	336
Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln	
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GGG ACC ACG GTC ACC GTC TCC TCA	360
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116 121	

(2) INFORMATION FOR SEQ ID NO:2:

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(B) TYPE: amino acid

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 21 26 31
 Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
 36 41 46
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 51 56 61
 Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
 66 71 76 81
 Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
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- (D) OTHER INFORMATION: /product= "HP1/2 light chain variable region"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /note= "pBAG172 insert: HP1/2 light chain variable region"

-29-

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35 40 45	
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GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCG TAC	288
Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr	
85 90 95	
ACG TTC GGA GGG GGG ACC AAG CTG GAG ATC	318
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile	
100 105	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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1 5 10 15	
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp	
20 25 30	
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile	
35 40 45	

-30-

Tyr	Tyr	Ala	Ser	Asn	Arg	Tyr	Thr	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly
50						55					60				
Ser	Gly	Tyr	Gly	Thr	Asp	Phe	Thr	Phe	Thr	Ile	Ser	Thr	Val	Gln	Ala
65					70					75					80
Glu	Asp	Leu	Ala	Val	Tyr	Phe	Cys	Gln	Gln	Asp	Tyr	Ser	Ser	Pro	Tyr
				85					90					95	
Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile						
			100					105							

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CLAIMS:

1. A method for the treatment of asthma comprising administering to a mammal suffering from asthma a composition comprising an anti-VLA-4 antibody.
2. The method of Claim 1, wherein the anti-VLA-4 antibody composition is administered intravenously.
3. The method of Claim 1, wherein the anti-VLA-4 antibody composition is administered in the form of an aerosol by inhalation.
4. The method of Claim 1, wherein the anti-VLA-4 antibody is selected from HP1/2, HP2/1, HP2/4, L25, and P4C2.
5. The method of Claim 1, wherein the anti-VLA-4 antibody is HP1/2, or a fragment thereof capable of binding to VLA-4.
6. The method of Claim 1, wherein the composition is administered at a dosage so as to provide from 0.05 to 5.0 mg/kg of antibody, based on the weight of the asthma sufferer.
7. The method of Claim 6, wherein the composition is administered at a dosage so as to provide 0.5 to 2.0 mg/kg of antibody, based on the weight of the asthma sufferer.
8. The method according to Claim 1, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 10 μ g/ml.
9. The method of Claim 1, wherein the composition is administered prior to exposure to an allergen to which the asthma sufferer is hypersensitive.
10. The method of Claim 1, wherein the mammal is a human.

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11. The method of Claim 1, wherein the composition is administered after exposure to an allergen to which said mammal is hypersensitive.

12. A method for the treatment of asthma comprising administering to a mammal suffering from allergic asthma an antibody, a recombinant antibody, a chimeric antibody, fragments of such antibodies, a polypeptide or a small molecule capable of binding to the α_4 subunit of VLA-4, or combinations of any of the foregoing, in an amount effective to provide inhibition of late phase response to an allergen to which the sufferer is hypersensitive or to provide decreased airway hypersensitivity in said mammal following allergen challenge.

13. The method of Claim 12, wherein the antibody, polypeptide or molecule is selected from monoclonal antibody HP1/2; Fab, Fab', F(ab')₂ or F(v) fragments of such antibody; soluble VCAM-1 polypeptides; or small molecules that bind to the VCAM-1-binding domain of VLA-4.

14. The method of Claim 12, wherein the composition comprises a plurality of anti-VLA-4 monoclonal antibodies or VLA-4-binding fragments thereof.

15. The method of Claim 12, wherein the composition includes, in addition to anti-VLA-4, an anti-ELAM-1 antibody, or an anti-ICAM-1 antibody, or both anti-ELAM-1 and anti-ICAM-1 antibodies.

16. The method of Claim 12, wherein the anti-VLA-4 antibody is HP1/2, or a fragment thereof capable of binding to VLA-4.

17. The method of Claim 12, wherein the composition is administered at a dosage so as to provide

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from 0.05 to 5.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

18. The method of Claim 17, wherein the composition is administered at a dosage so as to provide 1.0-2.0 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the asthma sufferer.

19. The method according to Claim 12, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 10 μ g/ml over a period of 7 days.

20. A pharmaceutical composition effective to attenuate late phase response or significantly reduce airway hypersensitivity in an asthmatic mammal consisting essentially of a monoclonal antibody recognizing VLA-4 in a pharmaceutically acceptable carrier.

1/6

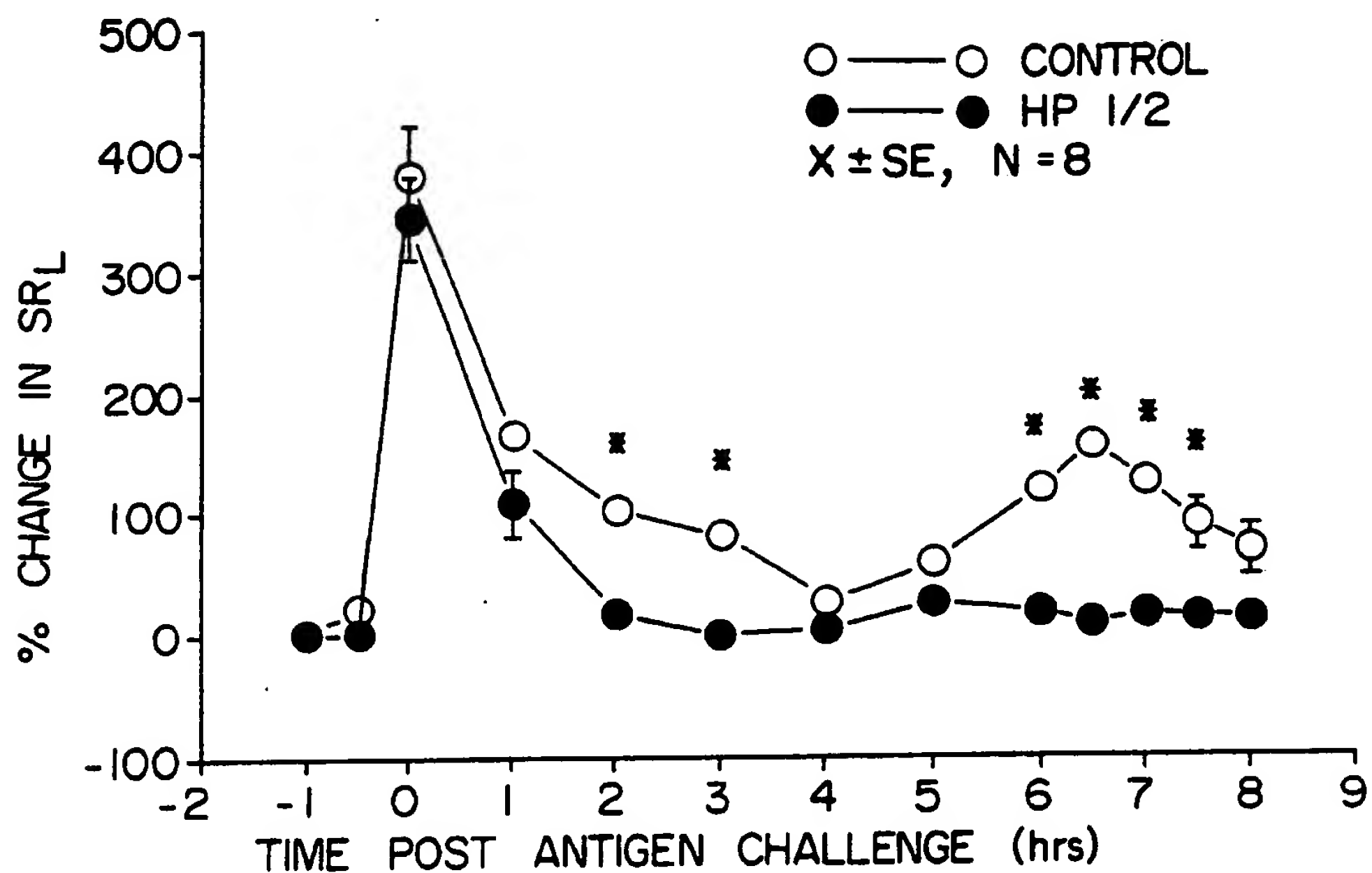


FIG. 1

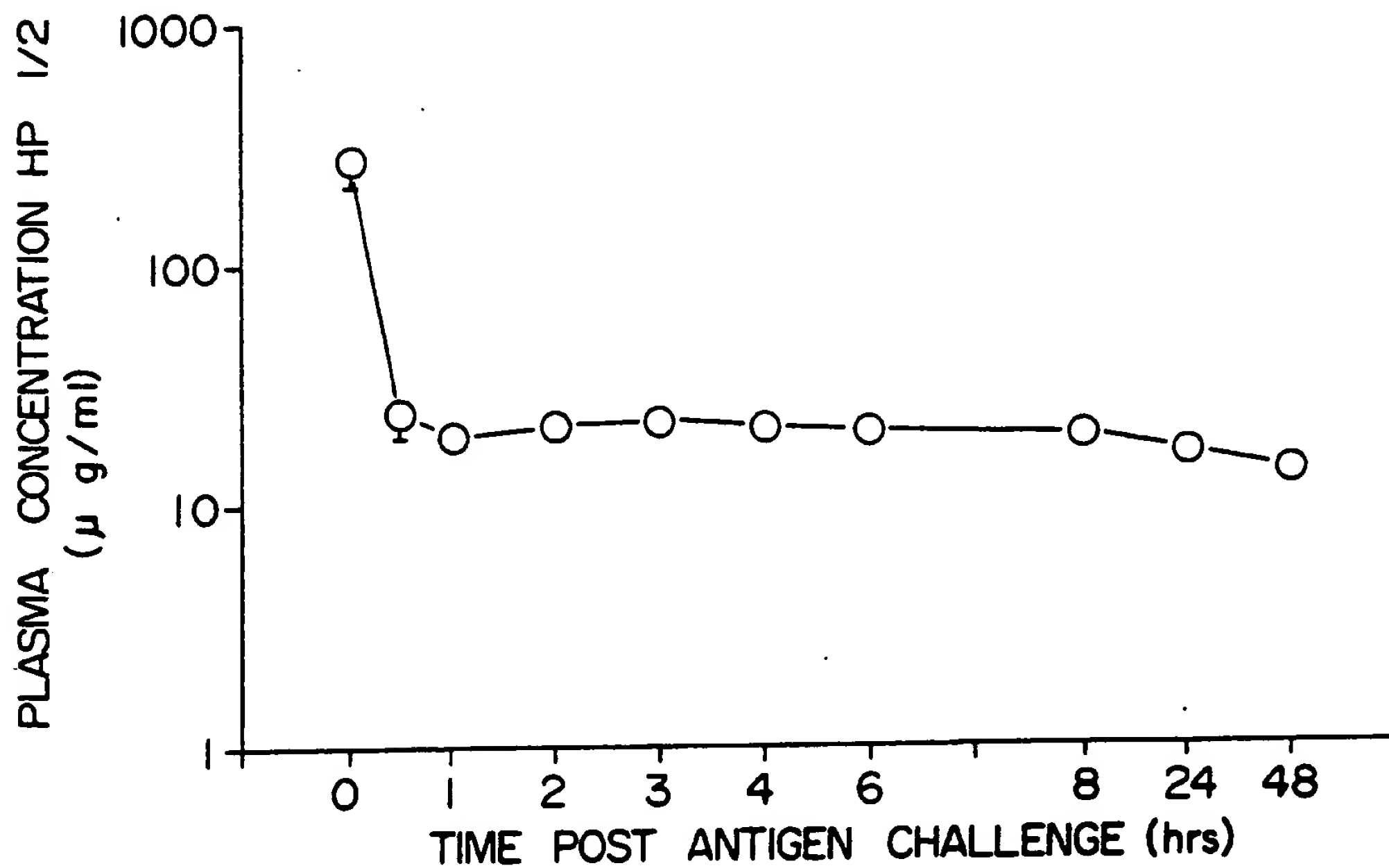


FIG. 2

SUBSTITUTE SHEET

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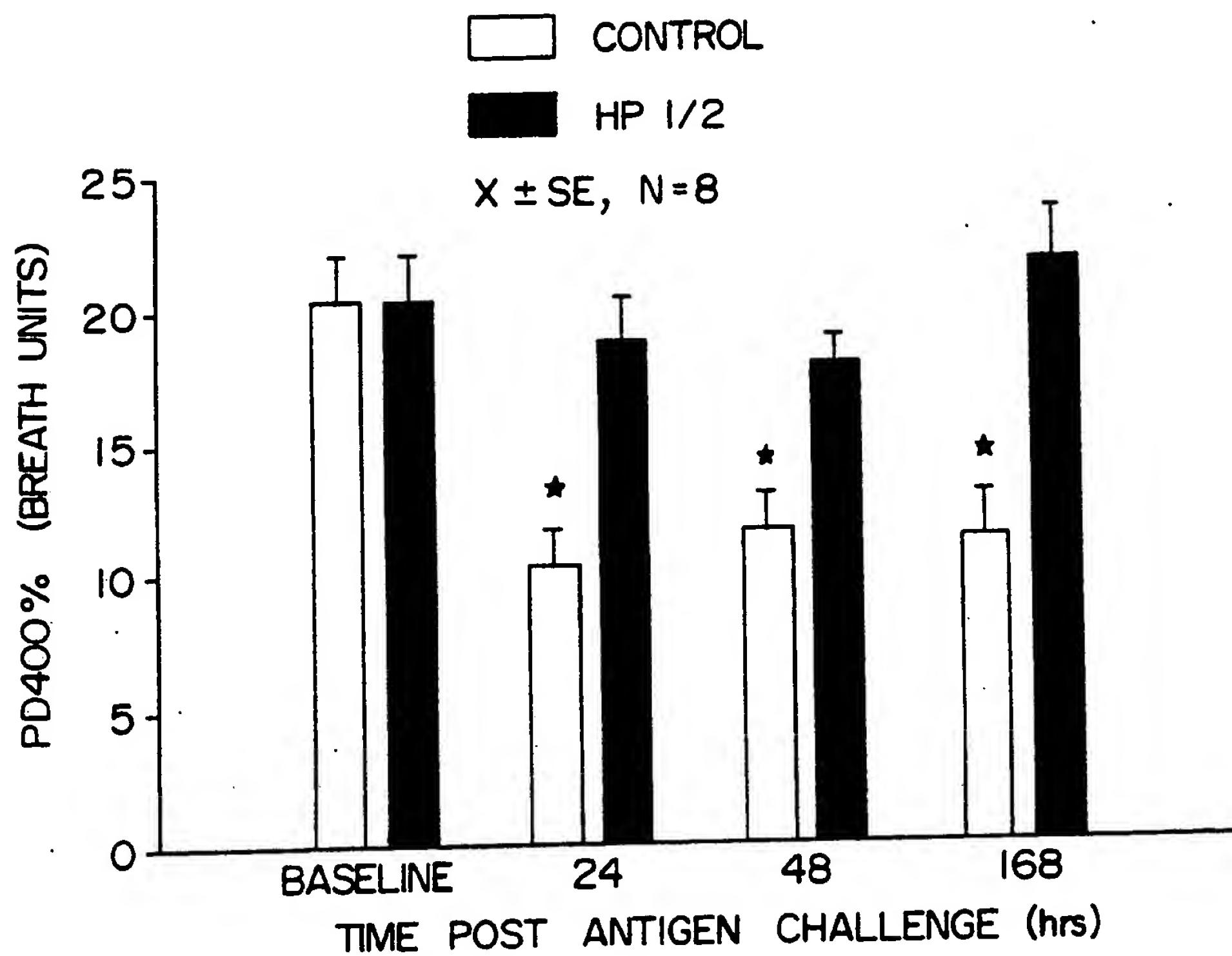


FIG. 3

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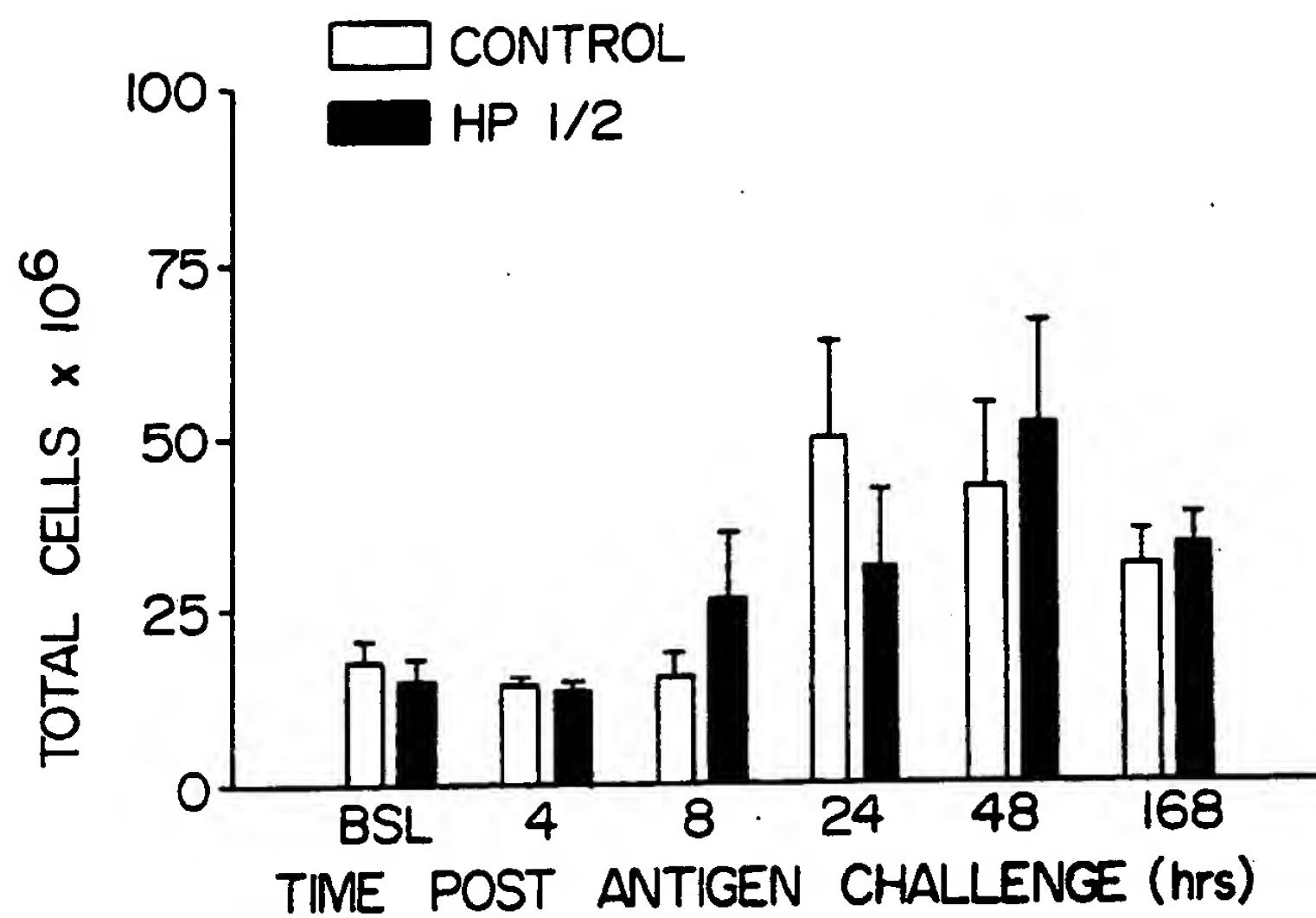


FIG. 4A

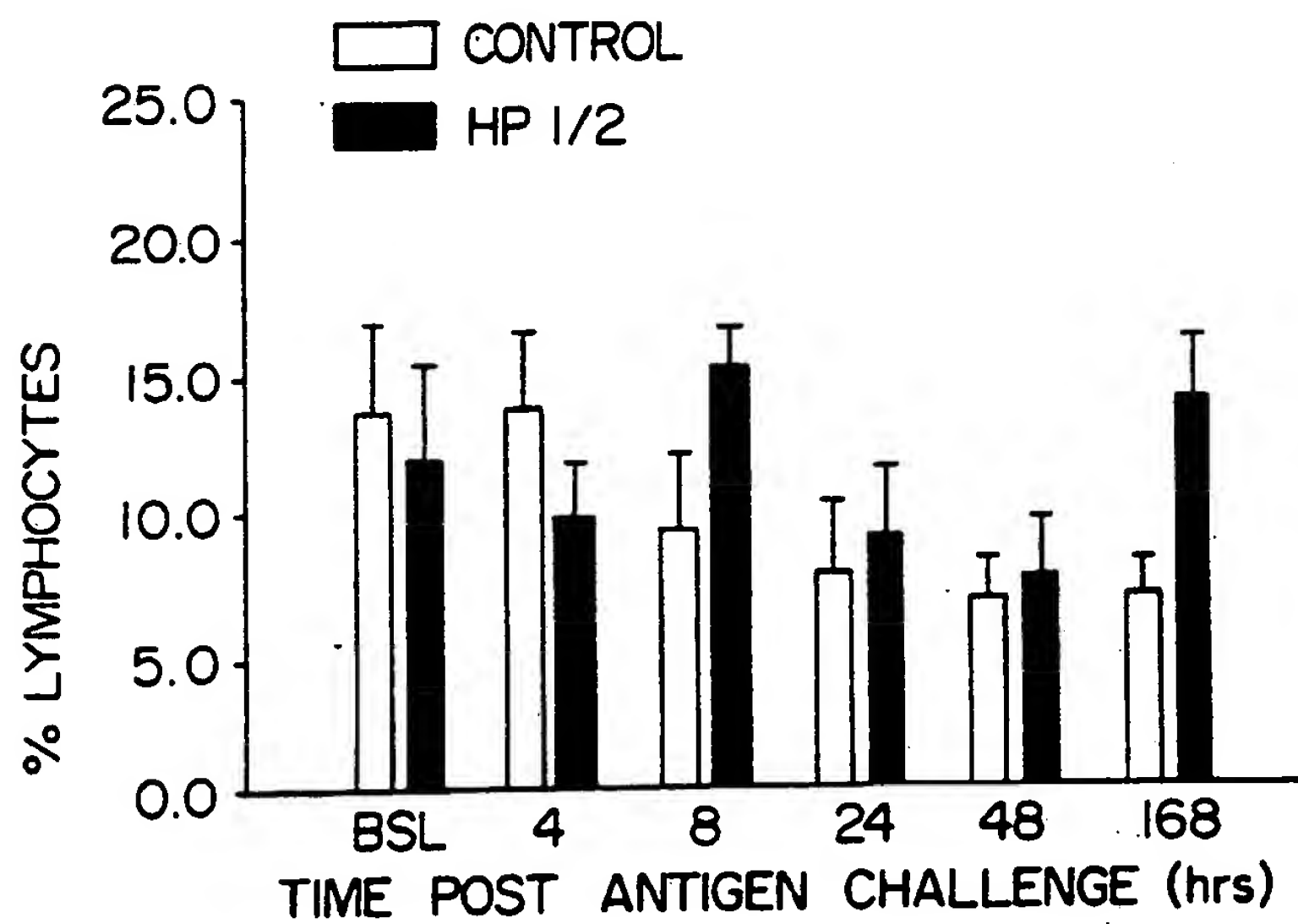


FIG. 4B

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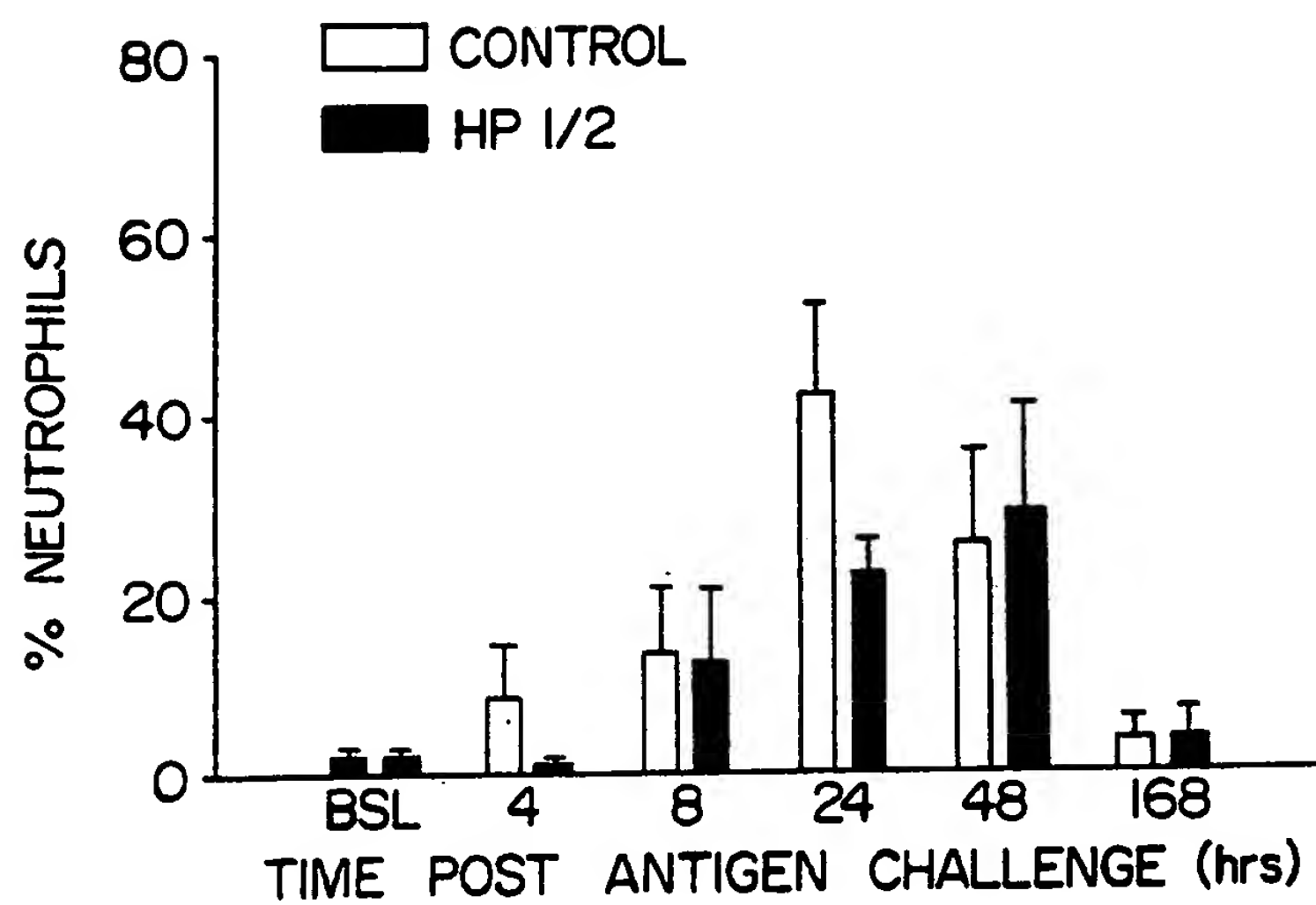


FIG. 4C

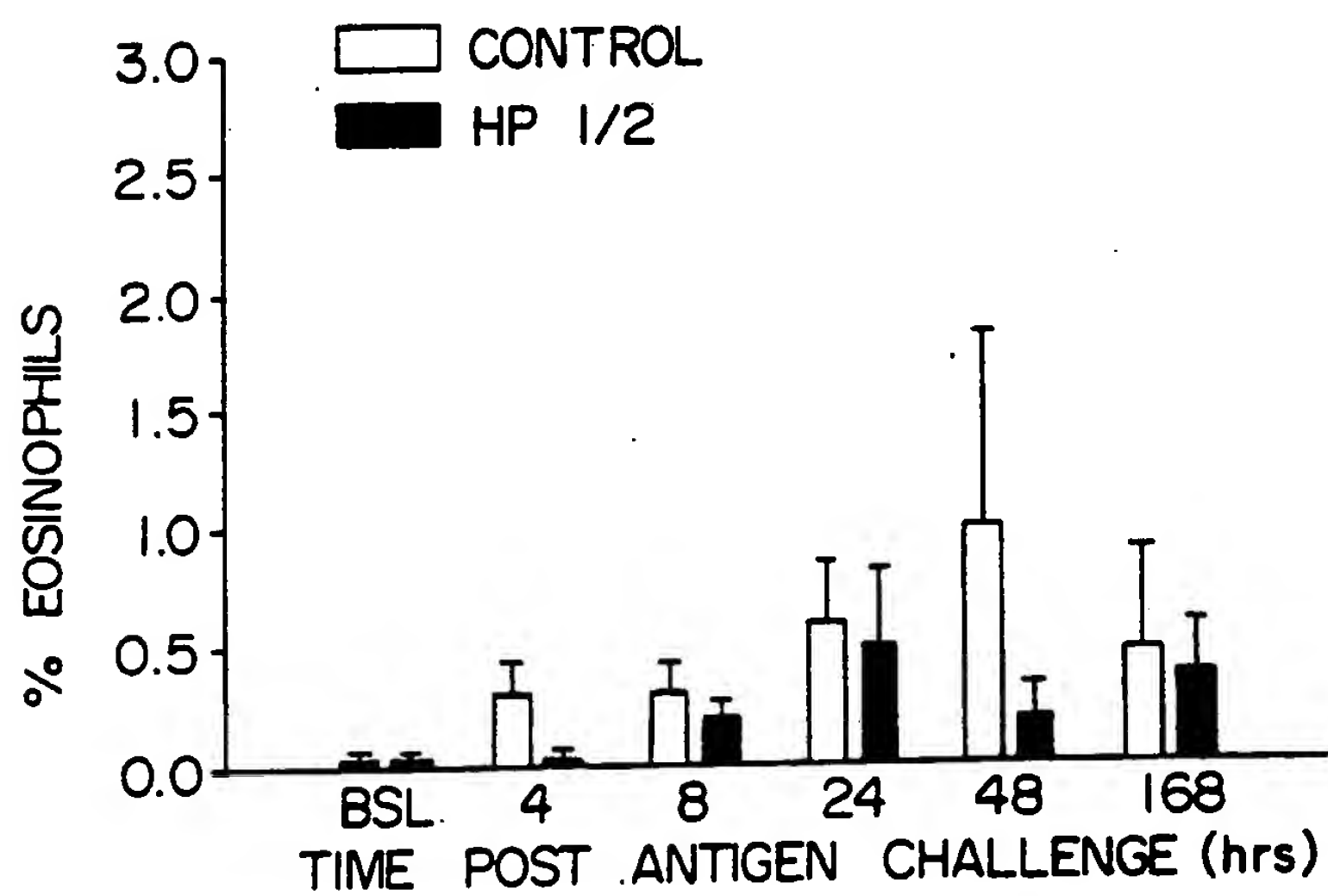
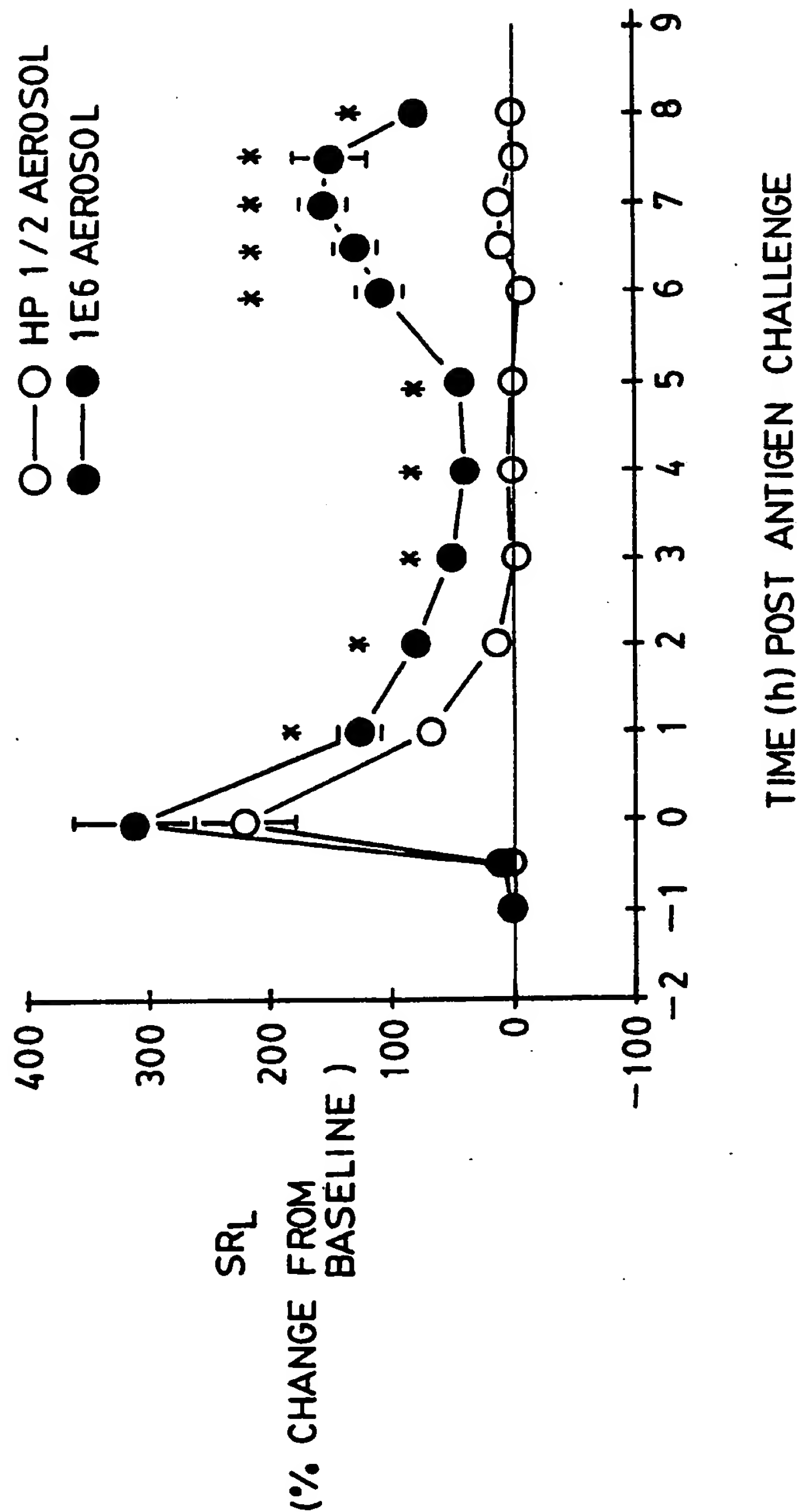


FIG. 4D

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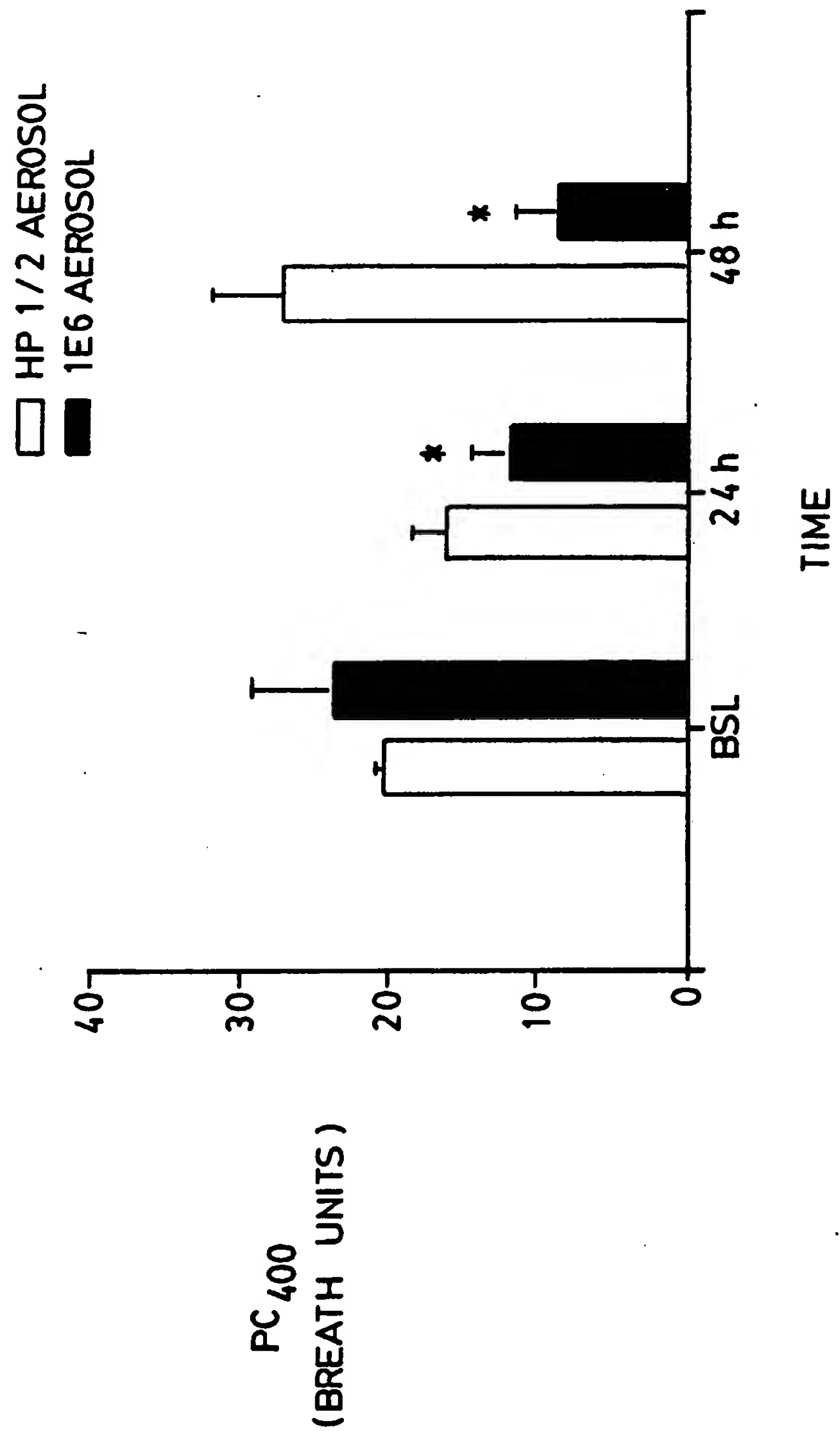
FIG. 5



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6/6

6/6

FIG. 6



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/00030

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁵ : A 61 K 39/395		
II. FIELDS SEARCHED		
Minimum Documentation Searched ?		
Classification System	Classification Symbols	
IPC ⁵	A 61 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT †		
Category *	Citation of Document, † with indication, where appropriate, of the relevant passages ‡	Relevant to Claim No. ‡
X	EP, A2, 0 330 506 (DANA-FABER CANCER INSTITUTE) 30 August 1989 (30.08.89), claims.	20
P, X	WO, A1, 92/00 751 (NOVO NORDISK A/S) 23 January 1992 (23.01.92), claims 1, 7, 10-15, 20, 24, 25, 27.	20
A	CHEMICAL ABSTRACTS, vol. 115, no. 23, issued 1991, December 09 (Columbus, Ohio, U.S.A.), S. PARMENTIER et al. "Role of glycoprotein IIa (beta1 subunit of very late activation antigens) in platelet functions", page 539, the abstract-no. 252 791h, Blood 1991, 78(8), 2021-6.	20
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: †</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"G" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
11 May 1993	01-06-1993	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	SCHNASS e.h.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 114, no. 9, issued 1991, March 04 (Columbus, Ohio, U.S.A.), R. ONISH et al. "A monoclonal antibody, 2H7, which defines a new very late activation antigen, inhibits IL-2-mediated cell proli- feration", page 545, the abstract-no. 79 730s, Nippon Ketsueki Gakkai Zasshi 1990, 53(6), 951-63.	20
A	<p>---</p> CHEMICAL ABSTRACTS, vol. 106, no. 5, issued 1987, February 02 (Columbus, Ohio, U.S.A.), H.G. BLUESTEIN et al. "Immunopathogenesis of the neuropsychiatric mani- festations of systematic lupus erythematosus", page 395, the abstract-no. 31 234r, Springer Semin. Immunopathol. 1986, 9(2-3), 237-49.	20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/00030

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:
Article 17(2)(b) & Rule 39.1(iv) PCT
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/US 93/00030 SAE 68912

In diesem Anhang sind die Mitglieder
der Patentfamilien der im obenge-
nannten internationalen Recherchenbericht
angeführten Patentdokumente angegeben.
Diese Angaben dienen nur zur Unter-
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family
members relating to the patent documents
cited in the above-mentioned inter-
national search report. The Office is
in no way liable for these particulars
which are given merely for the purpose
of information.

La présente annexe indique les
membres de la famille de brevets
relatifs aux documents de brevets cités
dans le rapport de recherche inter-
national visée ci-dessus. Les renseigne-
ments fournis sont donnés à titre indica-
tif et n'engagent pas la responsabilité
de l'Office.

In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 330506	30-08-89	EP A3 330506 JP A2 2003700	20-06-90 09-01-90
WO A1 9200751	23-01-92	AU A1 82055/91 DK A0 1628/90	04-02-92 06-07-90